HEALING PATTERN OF REAMED BONE FOLLOWING BONE HARVESTING BY A RIA DEVICE

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Abstract

Intramedullary nailing has been used for decades to treat fractures of the long bones. However, complications related to the increase in medullary pressure culminated in the development of the Reamer Irrigator Aspirator (RIA). Since its first clinical use, the RIA has moved from a reaming device to a cell and autologous bone-harvesting tool. This increase in use brings with it further clinical questions; namely, does the endosteal bone regenerate sufficiently to allow subsequent reaming procedures.

In the current study, endosteal bone regeneration post reaming was assessed in an ovine model. The study included six animals that had one tibia reamed, while the contralateral tibia acted as an intact control. Animals were administered fluorochrome labels in vivo, and bone regeneration was assessed using radiographical analysis. The endpoint of the study was 12 weeks post-surgery, at which time ex vivo analysis consisted of computed tomography and histological assessments.

In vivo radiographs indicated limited healing of the reamed bone. However, ex vivo computer tomographical analysis indicated no significant differences in terms of bone volume between the reamed bone and the intact bone. Histological assessment of these regions indicated new bone formation. Fluorescent labelling indicates strong bone formation from 9 weeks post-surgery and as such, the bone formed at 12 weeks was immature in nature and was actively undergoing remodelling.

These results indicate that bone regeneration post-reaming was continuing at three months. Therefore, given more time it may have sufficiently healed to allow a surgeon to use the intramedullary canal for a re-reaming procedure.

Keywords: RIA, reaming, bone healing, intramedullary nailing, sheep.

Background

The Reamer Irrigator Aspirator (RIA) was designed to allow the simultaneous suctioning and irrigation of the medullary canal while reaming for the placement of an interlocking nail. The RIA process results in the harvesting of a large amount of bone, which can be collected separately in a dedicated filter system. Consequently, the RIA has been used in clinical situations for autologous bone harvesting for the treatment of large bone defects. However, the endosteal healing pattern of the reamed bone has not been studied.

Introduction

Intramedullary nailing has been used for decades to treat fractures of the long bones (Muller et al., 2006; Pfeifer et al., 2010) and is the method of choice for most displaced adult diaphyseal fractures of the femur and tibia (Frolke et al., 2004). Intramedullary nailing can be performed using small diameter nails, which are placed directly into the intramedullary canal, or alternatively large diameter nails following a reaming procedure. In this reaming procedure, a cutting device is inserted into the intramedullary canal and bone is removed from the inner cortex of the bone. Prospective clinical studies have shown that long bone fractures, stabilised using an intramedullary nail, heal more quickly following reaming compared to non-reamed bones (Frolke et al., 2004; Porter et al., 2009). Yet, Högel et al. (Hogel et al., 2011) has shown that there is a decrease in callus formation in conventionally reamed limbs compared to intact limbs. Nevertheless, complications arising from the use of reaming devices have been well established and were first identified by Küntscher after his focused approach had led to its widespread use (Hammer et al., 2007). These complications are related to the increase in medullary pressure during reaming which causes a systemic embolisation of the medullary cavity contents into the venous system leading to the obstruction of nutritive arteries and transcortical blood vessels by the medullary contents in particular fat (Hogel et al., 2011; Husebye et al., 2011; Muller et al., 2006).

To alleviate these complications, Danckwardt-Lillieström (Danckwardt-Lilliestrom, 1969) – cited in Green (2010) – created a suction/irrigation method used during reaming to reduce this intramedullary pressure. However, it was not until the 1990s that a clinically applicable device was developed, which culminated in the development of the Reamer Irrigator Aspirator (RIA) by Synthes Inc (West Chester PA, USA) and was first used surgically in January 2001 (Green, 2010). It did not take
researchers and clinicians long to realise that the RIA system had further uses than merely reaming the bone. Its application has been investigated for the treatment of intramedullary infections (Zalavras and Sirkin, 2010), the collection of autologous bone grafts (Beck et al., 2013; Frolik et al., 2004; Hammer et al., 2007; Newman et al., 2008), and more recently the collection of cells (Henrich et al., 2010; Porter et al., 2009). In all cases, the RIA has shown promising results. Indeed, for autograft collection its use is indicated in cases where insufficient iliac crest bone is available following an initial surgery (Newman et al., 2008). Nevertheless, the surgeon should be careful not to over-ream the intramedullary canal as this can also lead to complications (Belthur et al., 2008).

While there have been clinical papers in relation to re-harvesting from cancellous beds (Moed et al., 1998; Papadopoulos et al., 2009; Conway, 2010; Qvick et al., 2013), the question whether or not the endosteal bone regenerates after reaming remains unanswered. Therefore, the current study was designed to address the question if a patient required additional treatments following bone harvesting with RIA, would it be possible to re-harvest the intramedullary canal? In this study, it was hypothesised that the endosteal bone surface does regenerate following reaming with a RIA and as such this would be possible.

Materials and Methods

Six female Swiss alpine white sheep, aged between two and four years old, were included in this study under the approval of the local governmental animal use committee (Graubünden TVB 08/2009). While it is known that bone repair is faster in young animals compared to elderly humans, the bone healing observed in this age range of sheep would be indicative of bone repair in a young human. They were assessed to be healthy and free of orthopaedic disease, based on physical examination prior to enrolment in the study. Cranial-caudal and medial-lateral radiographs of the tibia were taken prior to surgical interventions to ensure that the intramedullary canal was sufficiently large to accommodate the RIA reamer heads and that the bone was as straight as possible.

The sheep were housed initially as a group at the animal facility for 2 weeks. One week prior to surgery, the sheep were placed in individual boxes with close visual, olfactory, and auditory contact with other sheep for the duration of the study.

Operative procedures

Prior to surgical intervention, either the left or the right hind limb of each animal was randomly selected for reaming. Subsequently, each selected tibia was reamed with a RIA device under fluoroscopic guidance, using progressively smaller reamers to avoid injury to the outer cortex of the tibia – using procedures outlined by Beck et al. (Beck et al., 2013). Reaming progressed distally into the isthmus of the tibia up to a mean distance of 11.8 cm (± 2.5 cm). Sheep anaesthesia and analgesia were performed using standard protocols, similar to those used in previous work (Devine et al., 2009). Namely, animals were sedated using Detomidine (Domosedan®, 0.05 mg/kg, Intramuscular (IM)). General anaesthesia was induced using Diazepam (Valium®, 0.2 mg/kg, Intravenous (IV)) and Ketamine (Ketasol-100®, 4 mg/kg, IV), and was maintained with isoflurane (approx. 2 % in oxygen: oxygen flow rate of 0.6-1 L/min). Analgesia was administered preoperatively using Carprofene (Rimadyel®, 4 mg/kg IV) and epidural anaesthesia using 2 mg/kg Lidocaine plus 0.005 mg/kg Buprenorphine. Postoperative analgesia consisted of Carprofene (4 mg/kg Subcutaneous (SC)) for 5 days and Fentanyl Patches (Durogesic® Matrix, 75 μg/h) 150 μg/h for 72 h. Prophylactic antibiotics (Ampicillin, 10 mg/kg, IM) were administered at induction and 12 h postoperatively.

For surgical intervention, the sheep were placed in dorsal recumbency and the stifle and tibia were aseptically prepared and draped in a routine manner for an approach to the proximal tibia. An 8 cm parapatellar skin incision was made medially over the stifle and proximal tibia, and the fascia was incised. The incision was made down through the joint capsule over the craniomedial aspect of the proximal tibia. The stifle was placed in flexion and a 4 mm pointed Steinmann pin, on a hand chuck, was placed on the proximo-cranial aspect of the tibial tuberosity, just cranial to the medial meniscus. The pin was used to penetrate the cortex and enter the medullary canal. The dedicated RIA drill bit was used to enlarge the cortical hole. A 2.5 mm guide wire was then introduced into the canal and inserted to the most distal aspect of the tibia. Positioning was confirmed with intra-operative fluoroscopy. Reaming was performed under fluoroscopic guidance, using a 15 mm cutting head to open up the metaphyseal bone. Reaming was progressed distally using progressively smaller diameter reamers to a final 12 mm cutting head to allow reaming of as much of the tibia as possible, while ensuring that no damage occurred to the outer cortex of the bone (Fig. 1). For irrigation, sterile saline was drawn from an

![Fig. 1. Intra-operative fluoroscopy showing the 2.5 mm guide wire extended to the distal aspect of the tibia, and a 12 mm guide wire in the medullary canal.](image-url)
intravenous bag via the tube assembly manifold. Upon completion of the reaming procedure, the para-patellar fascia was closed with 0 PDS simple continuous pattern and the subcutaneous tissue and skin were closed in two layers in a routine manner using 2-0 Polydioxanone Suture PDS. Skin sutures were placed and an adhesive bandage was placed over the incision.

Post-operative procedures
Post-operatively, the sheep were maintained in individual pens for two weeks, then group-housed for the remainder of the study.

In vivo new bone formation was labelled intra-vitally with bone marker fluorochromes. Calcein green (10 mg/Kg, SC) was administered three weeks post operatively, Xylenol orange (90 mg/Kg, SC) was administered six weeks post operatively and tetracycline (Engemycin 30 mL per animal, SC) was given at nine weeks post operatively. These markers bind to calcium and therefore adhered to freshly formed bone on the day of injection.

Radiographs of the operated tibia were taken after surgery and monthly thereafter. The final radiographs were taken immediately post mortem. Radiographs were used to select the regions of interest for computed tomography (CT) scanning, based on indentation of the cortex post-operatively.

**Post-mortem analysis**
A twelve week end point was selected in this study, as it was sufficient time for the limb to show signs of healing – since a fracture of the tibia heals within 12 weeks (Zeiter *et al.*, 2004). Furthermore, this is early enough to allow histological assessment of bone formation and remodelling as it occurred. Therefore, twelve weeks after surgery, the animals were euthanised by means of an intravenous overdose of barbiturate (pentobarbital, Esconarcon® 60 mg/kg, IV). Both tibiae were removed from the body and the soft tissue cut from the bone immediately after euthanasia. Both tibiae were fixed in 70% ethanol for computed tomography imaging and histological assessments.

**High resolution peripheral quantitative computed tomography**
Each tibia underwent ex vivo high-resolution three-dimensional peripheral quantitative computed tomography (HR-pQCT) scan. The HR-pQCT X-ray tube (XtremeCT Scanco Medical, Brüttisellen, Switzerland) was operated at 60 kVp, 900 μA). Scans were performed at 82 μm isotropic resolution in two different regions of interest (ROI; Fig. 2). The first ROI in the metaphyseal bone in the proximal tibia (M) had a length of 3 cm which began 2 cm distal to the upper tibia extremity. The distal ROI in the diaphyseal tibia (D) also had a length of 3 cm. However, in the case of

Fig. 2. Methaphyseal (yellow) and diphyseal (red) locations of the regions of interest chosen on the radiograph of sheep number 3 for CT scanning and histological sectioning.
the distal ROI, this was taken as the lowest reamed point that could be identified by post-surgery radiographs and as such its location varied between specimens. Despite this, sheep have a relatively short transitional zone between trabecular and cortical regions compared to humans and, as such, the D-ROI for each animal was located in cortical diaphyseal bone. Identical ROI were selected for the un-operated contralateral tibia.

Bone tissue was segmented using two different thresholds. The range of [206-3,000] mg hydroxyapatite (HA)/cm³ was used to detect the total amount of bone considering both cortical and trabecular bone, while the range of [206-656] mgHA/cm³ was used to detect only new bone formation.

Parameters of interest were the volume of the mineralised tissue (Bone volume BV in mm³) and the volumetric bone mineral density (BMD in mgHA/cm³).

**Histological processing**

Contact radiographs of the entire specimens were taken using a cabinet X-ray system (model 43855A, Faxitron X-ray Corporation) prior to histological embedding. Samples were fixed in 70 % ethanol for 6-10 weeks. The samples were dehydrated in an ascending alcohol series, placed in xylene for 1-5 days prior to infiltration with and embedding in methyl methacrylate (MMA).

The samples were trimmed with a band-saw (Bizerba AG, Zurich, CH) to remove excess MMA so that they would fit into a Leica 1600 saw (Leica AG, Glattbrugg, CH). Serial cross sections approximately 200 µm thick were prepared from the same ROIs used for computed tomography scanning. Contact radiographic images were taken of each section to select representative sections. These sections were glued using cyanoacrylate onto Plexiglas holders, ground and polished to a thickness of 60-100 µm using a Micro Grinding System (Exact, Norderstedt, GER).

For evaluation of new bone formation over time, analysis with fluorochromes was performed using unstained sections. Additional sections were surface stained with Giemsa-Eosin for histological assessment.

**Statistical analysis**

When testing a novel hypothesis it is difficult to predict the relative difference between groups. However, for the results to be clinically relevant an effect size of 1.8 was
selected for the current study. Using this effect size it would not be possible to detect small differences between limbs. However, these small differences would have little clinical relevance. Using this method, it was predicted that 6 sheep would enable the detection of statistical differences between limbs at a \( p \) value of 0.05 with a power of 80%. Hence, six animals were enrolled in the current study design. The reamed and the intact data were tested for normality. For statistical analysis, a paired student-\( t \)-test was utilised to compare the reamed and unreamed bone using statistical software (Minitab version 16, Minitab Inc. USA). Differences between groups were considered significant when \( p \leq 0.05 \).

Results

No surgical-related or post-surgical complications were recorded during this study. Each tibia was reamed to a depth as defined by the surgeon based on intraoperative fluoroscopic guidance (Fig. 3). It was found that the length of bone which was reamed was dependent on the curvature of the tibia and varied with each animal. In this study a mean (± Standard Deviation; SD) reaming depth of 11.8 cm (± 2.5 cm) was used.

In vivo bone formation was assessed using monthly radiographs. From these radiographs no in vivo complications were observed. In vivo radiographs did indicate some bone formation, however the most distal point of reaming was clearly visible at all-time points analysed (Fig. 4).

From HR-pQCT data (Table 1), it was found that in the D-ROI there was no statistically significant difference in the total bone volume (TBV) of the reamed and contralateral tibiae (\( p = 0.643 \)). However, there was a significant increase in new bone volume (NBV) in the reamed limbs in this region (\( p = 0.035 \)). This increase in NBV in the reamed limbs corresponded with a statistically significant decrease in total bone density (TBD) in comparison to the contralateral intact limb. In the M-ROI, no statistically significant differences between the reamed and contralateral tibiae were detected in TBV, TBD or NBV (\( p \geq 0.358 \)). No differences were detected in the new bone density in either ROI (\( p \geq 0.626 \)).

Histological analysis of the intact bones showed a bone marrow cavity predominantly consisting of large amounts of univacular white fatty tissue (Fig. 5a). There was a slight to moderate number of trabeculae in the M-ROI. However, there were no trabeculae in the D-ROI. In contrast, the reamed tibia had slight to moderate formation of mesenchymal fibrous connective tissue recorded predominantly in the M-ROI (\( n = 6 \) animals), which indicates reparation of the iatrogenic insult. Additionally, a minimal to slight deposition of hemosiderin in reamed areas indicated a residue of previous haemorrhages (\( n = 5 \) animals). The reamed bone marrow cavity predominantly consisted of large amounts of univacular white fatty tissue. In all animals, there were clear signs of new bone formation at the endosteal area of the D-ROI (Fig. 5b), but this varied between animals from minimal to moderate. Furthermore, there was an increase of bone remodelling in

![Fig. 5. Photomicrograph overview of Tibia, (a) Control medullary cavity in the D-ROI, (b) reamed medullary cavity of the contralateral limb – indicating bone formation had occurred at 12 weeks post-surgery (scale bar = 2 mm). (c) Higher magnification (scale bar = 500 \( \mu \)m) of selected region in b illustrating ongoing bone formation and the immature nature of the newly formed bone. Additionally, bone remodelling within the cortex was observed (arrows). (d) Depicts a fluorescent image of the same region in a consecutive section to c, illustrating a strong tetracycline fluorescence which indicates that bone regeneration primarily occurred following six post-surgical weeks. “*” indicates bone which was likely formed following TC administration. Giemsa-Eosin.](cellsmaterials)
healing pattern of reamed bone following bone harvesting by a RIA device

Harvesting autologous bone material (Beck technique) was easy and reliable and effective for work, it was found that collection of RIA material using the assessment of healing at 3 months. Similar to previous removal sufficient to enable a radiological and histological Nevertheless, the method used produced a zone of bone reaming process, the surgeon passed the reamer head into the intramedullary canal, as the tip of the RIA is rigid and completely inflexible. Therefore, in the current study a large reamer was used for the initial insertion into the bone, followed by the use of a smaller reamer along the mid-shaft of the bone to overcome this issue. During the reaming process, the surgeon passed the reamer head into the tibia intramedullary canal under fluoroscopic guidance, to maximise the reaming distance safely permissible without risking damage to the outer cortex of the tibia. This is in order to replicate the human situation, where the surgeon attempts to collect the maximum volume of bone for transplantation. This lead to variations in the location of the D-ROI.

Discussion

It has been reported that the two essential factors required for the successful osseous healing are good stability and blood supply (Rhinelander, 1974 – cited Reed et al., 2002). Therefore, it may be expected that the RIA reaming process used for the collection of bone or cells from the medullary canal would affect endosteal bone formation as this process has been shown to damage bone vascularity (Klein et al., 1990). Incidental reports strongly suggest that diaphyseal regeneration does happen, at least in some instances (Conway, 2010; Qvick et al., 2013). However, this needs to be scientifically confirmed in a controlled experiment.

In this study, the in vivo radiographs did not indicate any major new bone formation during the study. However, radiographs are only capable of detecting highly mineralised bone and it is possible that new bone did form, but was not sufficiently mineralised to detect using this technique. In clinical practices, the diameter of the bone dictates the diameter of the reamer head used. However, due to the size restrictions of the sheep tibia and smallest size of the RIA, it was not possible for the investigators to ream the entire length of the long bone as is done clinically. Nevertheless, the method used produced a zone of bone removal sufficient to enable a radiological and histological assessment of healing at 3 months. Similar to previous work, it was found that collection of RIA material using the technique described was easy and reliable and effective for harvesting autologous bone material (Beck et al., 2013).

The two chosen ROI were selected, so that the healing pattern of both the metaphyseal trabecular bone and the diaphyseal bone could be examined. Nevertheless, the primary function of the reaming procedure is to collect bone particles for transplantation (Beck et al., 2013).

In the current study, researchers attempted to collect bone reamings from sheep using equipment designed for humans. In human surgery, the femur is the bone of choice for harvesting endosteal bone reamings. However, in sheep it is more applicable to harvest bone from the tibia, as there is easier surgical access. Additionally, the length of the sheep femur is much shorter and the cortical thickness much smaller than that of the tibia and therefore the tibia is the bone of choice for reaming experiments in sheep (Hammer et al., 2007). However, the sheep tibia has a natural curvature that complicates access to the intramedullary canal, as the tip of the RIA is rigid and completely inflexible. Therefore, in the current study a large reamer was used for the initial insertion into the bone, followed by the use of a smaller reamer along the mid-shaft of the bone to overcome this issue. During the reaming process, the surgeon passed the reamer head into the tibia intramedullary canal under fluoroscopic guidance, to maximise the reaming distance safely permissible without risking damage to the outer cortex of the tibia.

This is in order to replicate the human situation, where the surgeon attempts to collect the maximum volume of bone for transplantation. This lead to variations in the location of the D-ROI.

It should be noted that it is difficult to evaluate the accuracy of the thresholds used when analysing new bone using HR-pQCT, due to limitations of the image quality. Nevertheless, from bone volume and bone

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<tr>
<th>Table 1. High-resolution peripheral quantitative computed (HR-pQCT) data.</th>
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<tr>
<td>Control tibia (Mean ± SD)</td>
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<tr>
<td><strong>Total Bone Volume (mm³)</strong></td>
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<tr>
<td>11850 ± 2104</td>
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<tr>
<td><strong>New Bone Volume (mm³)</strong></td>
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<tr>
<td>6421 ± 2674</td>
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<td><strong>Total Bone Density (mg HA/cm³)</strong></td>
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<tr>
<td>804 ± 89</td>
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<td><strong>New Bone Density (mg HA/cm³)</strong></td>
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<td>436 ± 10</td>
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* indicates significant differences between limbs.
density measurements using HR-pQCT, it was found that in the D-ROI there was no difference in the TBV of the reamed tibia in comparison to the contralateral tibia. However, when only new bone was considered, there was significantly more bone in the reamed tibia when compared to the intact tibia. This indicates that new bone formation has taken place to such an extent that there is no difference in the total bone in the D-ROI. Nevertheless, a considerable amount of bone in the reamed tibia is newly formed immature bone. This was further indicated by the fact that the TBD of the reamed tibia is significantly lower than that of the intact tibia, since new bone has a lower density than mature remodelled cortical bone. In the M-ROI no significant differences were detected in terms of bone volume or bone density in either ROI or using either threshold. In the M-ROI, trabecular bone predominates – the density of which would be similar to that of newly formed bone – which made separating these bones types by thresholding impossible. Furthermore, as no differences were detected between the TBD of the reamed and contralateral intact limbs, the maturity of the bone in the reamed and intact tibiae was similar.

Interpretations of the computed tomography were supported by the histological review of sections from both the D-ROI and the M-ROI. In all animals, there were signs of new bone formation in the endosteal area of the D-ROI. This bone was immature in nature, and active bone remodelling was observed in areas where new bone formation had occurred. Fluorochrome labelling indicated that this new bone formation primarily occurred later than 6 weeks post-surgery, as indicated by the relatively low levels of calcein green and Xylenol orange fluorescence. It is possible that these fluorochromes had been incorporated into the bone during early bone formation, but were lost during the remodelling phase. However, this is unlikely as the strong tetracycline fluorescence indicates that most of the bone regeneration occurred around or after this 9 week time-point. This indicates that time was needed to overcome the disruption to the endosteal vascular system before bone reparation could occur. The occurrence of remodelling within the intact cortex of the reamed tibia may also indicate that some degeneration of the surrounding cortical bone occurred due to a reduction in nutrition (Fig. 6b). However, this bone was undergoing repair and the regeneration of the iatrogenic insult was ongoing at 12 weeks and may well regenerate sufficiently in the future to enable the re-harvesting of bone from the same tibia, if the need arose.

Nonetheless, cortical thinning was observed radiographically even at 12 weeks post-surgery. This further indicated that bone regeneration was not yet sufficient for re-harvesting, at this time point. Due to this cortical thinning, subsequent harvesting procedures should be conducted with extreme care so as not to create further thinning of the cortex.

In addition, some bone fragments which appeared to have displaced to distal areas during reaming appeared viable at 12 weeks post-surgery. This would indicate that bone harvested during the reaming procedure is a viable source of autologous bone for use in other areas of the body.

It should also be noted that the animals utilised in this study were relatively young (comparable to young adults) and as such it is likely that their bone regenerative capacity would be greater than in elderly animals.

**Conclusion**

In conclusion, new bone formation has occurred in the reamed tibiae of sheep 3 months post RIA bone harvesting. The initiation of this bone reparation began more than 6 weeks post-reaming, due to the disruption of the endosteal vascular system. Nevertheless, the volume of bone formation at 12 weeks was comparable to that of the contralateral intact tibia, in both the diaphyseal and metaphyseal regions. However, this bone was immature and additional time would be needed before the bone returned to a normal state.

**Conflict of interest statement**

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**References**


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Discussion with Reviewers

Reviewer 1: The endocortex would seem to be rather stress protected. Do the authors have any comments on how Wolff’s Law relates to their findings?

Authors: From a mechanical point of view, a long bone could be considered as a thin walled cylinder. In thin walled cylinders, the circumferential stress exerted on the cylinder is inversely proportional to the wall thickness of the cylinder or in the case of a long bone the cortical thickness. As such, a decrease in the cortical thickness through reaming of the bone will increase the circumferential stress on the bone. Due to this increase in circumferential stress, the bone would adapt and remodel itself over time to compensate for this increase in accordance with Wolff’s law.